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## (54) Methods for generating polynucleotides having desired characteristics by iterative selective and recombination

(57) The present invention relates to a method for evolving a polynucleotide encoding a plurality of genes, e.g. multiple genes forming a multicomponent pathway. The method involves shuffling of polynucleotides by conducting a polynucleotide amplification process on overlapping segments of a population of variants of a polynucleotide encoding a plurality of genes under conditions whereby one segment serves as a template for extension of another segment to generate a population of recombinant polynucleotides. This population is screened for a recombinant polynucleotide encoding a plurality of genes having a desired property.

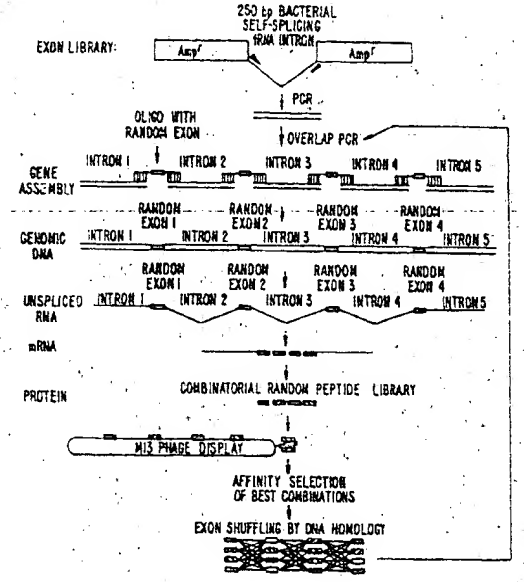


FIG. 20.

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Application Number
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## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int.Cl.6) |
|---|---|---|---|
| Y | WO 95 22625 A (AFFYMAX TECH NV ;STEMMER WILLEM P C (US); CRAMERI ANDREAS (US)) 24 August 1995 * the whole document * --- | 1-20 | C12N15/10 C12N15/64 C12Q1/68 //C07K14/435, C07K14/545, C12N9/86, C12N9/38, C12N15/52 |
| Y | STEMMER W: "DNA shuffling by random fragmentatio and reassembly: In vitro recombination for molecular evolution" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, October 1994, pages 10747-10751, XP002087463 * the whole document * --- | 1-20 | |
| Y | STEMMER W P C: "Rapid evolution of a protein in vitro by DNA shuffling" NATURE, vol. 370, 4 August 1994, pages 389-391, XP002082182 * the whole document * --- | 1-20 | |
| Y | W.P.C. STEMMER: "Searching sequence space" NATURE BIOTECHNOLOGY, vol. 13, June 1995, pages 549-553, XP002095510 NATURE PUBL. CO.,NEW YORK, US * the whole document * --- | 1-20 | TECHNICAL FIELDS SEARCHED (Int.Cl.6) C12N C12Q |
| Y | US 5 279 952 A (WU KUN C) 18 January 1994 * the whole document * --- | 1-20 | |
| Y | US 5 223 408 A (GOEDDEL DAVID V ET AL) 29 June 1993 * the whole document * --- | 1-20 | |
| | -/-- | | |

The present search report has been drawn up for all claims

| Place of search | Date of completion of the search | Examiner |
|---|---|---|
| THE HAGUE | 4 March 1999 | Hornig, H |

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## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int.Cl.6) |
|---|---|---|---|
| Y | R M HORTON ET AL: "Engineering hybrid genes without the use of restriction enzymes: gene splicing by overlap extension" GENE, vol. 77, 1989, pages 61-68, XP002090392 * the whole document * --- | 1-20 | |
| Y | HO S N ET AL: "SITE-DIRECTED MUTAGENESIS BY OVERLAP EXTENSION USING THE POLYMERASE CHAIN REACTION" GENE, vol. 77, no. 1, 1 January 1989, pages 51-59, XP000272761 * the whole document * --- | 1-20 | |
| A | WO 91 07506 A (US GOVERNMENT) 30 May 1991 * the whole document * --- | 1-20 | |
| A | HEIM R ET AL: "WAVELENGTH MUTATIONS AND POSTTRANSLATIONAL AUTOXIDATION OF GREEN FLUORESCENT PROTEIN" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, 1 December 1994, pages 12501-12504, XP000574454 * the whole document * --- | 1-20 | TECHNICAL FIELDS SEARCHED (Int.Cl.6) |
| P,X | A. CRAMERI ET AL.: "Improved green fluorescent protein by molecular evolution using DNA shuffling" NATURE GENETICS, vol. 14, March 1996, pages 315-319, XP002095449 NATURE PUBLISHING CO., NEW YORK, US * the whole document * --- | 1-16 | |
| | -/-- | | |

The present search report has been drawn up for all claims

| Place of search | Date of completion of the search | Examiner |
|---|---|---|
| THE HAGUE | 4 March 1999 | Hornig, H |

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## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int.Cl.6) |
|---|---|---|---|
| L | WO 97 35966 A (MAXYGEN INC ;MINSHULL JEREMY (US); STEMMER WILLEM P C (US)) 2 October 1997 * examples I-IV. * --- | 1-16 | |
| T | CRAMERI A ET AL: "Molecular evolution of an arsenate detoxification pathway y DNA shuffling" NATURE BIOTECHNOLOGY, vol. 15, May 1997, pages 436-438, XP002082183 * the whole document * ----- | 1-16 | |

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

The present search report has been drawn up for all claims

| Place of search | Date of completion of the search | Examiner |
|---|---|---|
| THE HAGUE | 4 March 1999 | Hornig, H |

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04-03-1999

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9522625 A | 24-08-1995 | US 5605793 A | 25-02-1997 |
| | | AU 2971495 A | 04-09-1995 |
| | | CA 2182393 A | 24-08-1995 |
| | | CN 1145641 A | 19-03-1997 |
| | | EP 0752008 A | 08-01-1997 |
| | | JP 10500561 T | 20-01-1998 |
| | | US 5811238 A | 22-09-1998 |
| | | US 5837458 A | 17-11-1998 |
| US 5279952 A | 18-01-1994 | NONE | |
| US 5223408 A | 29-06-1993 | US 5736135 A | 07-04-1998 |
| WO 9107506 A | 30-05-1991 | AU 6886991 A | 13-06-1991 |
| WO 9735966 A | 02-10-1997 | US 5837458 A | 17-11-1998 |
| | | AU 2337797 A | 17-10-1997 |
| | | AU 2542697 A | 17-10-1997 |
| | | WO 9735957 A | 02-10-1998 |
| | | AU 1087397 A | 19-06-1997 |
| | | CA 2239099 A | 05-06-1997 |
| | | EP 0876509 A | 11-11-1998 |
| | | WO 9720078 A | 05-06-1997 |

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